University of Szeged

Faculty of Pharmacy

Institute of Pharmaceutical Technology and Regulatory Affairs

Summary of PhD thesis

# DEVELOPMENT AND CHARACTERIZATION OF LORATADINE NANOSYSTEMS FOR INTRANASAL DELIVERY USING QUALITY BY DESIGN APPROACH

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# DEVELOPMENT AND CHARACTERIZATION OF LORATADINE NANOSYSTEMS FOR INTRANASAL DELIVERY USING QUALITY BY DESIGN APPROACH

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# 1. INTRODUCTION

Dosage forms design is associated with a great challenge to match the pharmacological/therapeutical expectations of the clinical practice with the attributes of the active pharmaceutical ingredients (API) and the biopharmaceutical environment of the targeted administration route. Poor aqueous solubility is one of the major challenges concerning the APIs in this complex development process within the task of pharmaceutical technology. Therefore, special interest can be seen on these Class II and IV drugs of the BCS.

Solubility enhancement and using alternative routes of administration are the main leading strategies to make these drugs available for the patients in several cases. The combination of the mentioned strategies is advised for drugs that have both weaknesses, namely (1) suffer from poor water solubility and (2) are extensively metabolized by the first-pass metabolism.

As a potential solution for the first challenge is nanosizing (nanotechnology) as the nanoscale-sized particles of an API exhibit higher solubility and dissolution rate compared to their large counterparts. It can be seen through the evaluating the new delivery pathways, that intranasal delivery has recently received a high interest as an alternative route of administration, as a promising route of administration for local, systemic, brain, and vaccine therapy. Moreover, combining these two strategies, one can lead to an innovative product, namely an intranasal nanosystems based formulation making the API available for both systemic and brain targeting.

Loratadine (LOR) is the most frequently prescribed antihistamine drug for the treatment of various allergic conditions. This API exhibits poor and variable bioavailability. Therefore, the delivery of LOR in a new dosage form based on a nanosized system could be advantageous to improve the bioavailability and introduce a new preparation of LOR of high patient acceptance.

From regulatory aspects, nanosystems form a distinctive group regarding their acceptance; relevant guidelines and relevant chapters of EMA and FDA must be applied during all manufacturing stages from material selection and formulation to the final production. Furthermore, the FDA has emphasized the application of the Quality by Design (QbD) methodology, which can be remarkably useful for the novel, high-risk dosage forms, and administration routes for careful planning and development even at the early phase of the research.

***Abbreviations:***

*AUC – Area under the curve; BCS – Biopharmaceutical classification system; Cd – Drug concentration; CQA – Critical quality attributes; CMP – Critical material parameters; CPP – Critical process parameters; DE – Dissolution efficiency; DLN – Dried loratadine nanoparticles'; DSC – Differential scanning calorimetry; EMA – European medicine agency; F68 – Pluronic F68; FDA – U.S Food and drug administration; FTIR – Fourier-transform infrared spectroscopy; HA – Sodium hyaluronate; HPMC – Hydroxypropylmethylcellulose; ICH – International council on harmonization; IN – Intranasal; J – Flux; Kp – Permeability coefficient; LNS – Loratadine nanosuspension; LOR – Loratadine; M – Mucin; MDT – Mean dissolution time; MPS – Mean particle size; NF – Nasal formulation; PDI – Polydispersity index; PM – Physical mixture; PVP-K25 – Polyvinylpyrrolidone K-25; QbD – Quality by Design; QTPP – Quality Target Product Profile; Tween 80 – Tween 80, poly(oxyethylenesorbitanmonooleate); RA – Risk assessment; REF – Reference; RD – Relative dissolution; SEM – Scanning electron microscopy; SLS – Sodium lauryl sulfate; TRE –Trehalose; XRPD – X-ray powder diffraction; ZP – Zeta potential.*

## 2. AIMS OF THE WORK

The main aim of this study was to develop a nanosystem-based intranasal formulation of LOR. Based on the literature background of the nasal delivery, nanosuspension was selected to prepare the pre-dispersion for the nasal formulation. The applicability of a nanosuspension in a nasal formulation is a new approach in pharmaceutical technology, therefore few data for such systems are available up till now. QbD approach was implemented to set the critical process and material parameters that impact the preparation of nanosuspensions. A nasal formulation containing the nanosuspension of the poor water-soluble LOR was developed as liquid formulations based on using a mucoadhesive agent. The nasal delivery of nanosystem-based LOR is a novel strategy that could improve the bioavailability of LOR and introduce a new dosage form with high patient acceptance.

Experimentally, the influential parameters were studied and optimized to develop the LOR nanosuspension as a pre-dispersion. For the final product, the concentrations of the drug and the mucoadhesive agent were investigated to finally evaluate the *in vitro* and *in vivo* characteristics of the prepared nanosuspension-based nasal formulation.

The main steps in the experiments were the following:

- Application of the extended QbD for research and development approach of nanosuspension as pre-dispersions containing LOR as H1 antihistamine agent.
- Selection of the pre-dispersion of LOR to formulate a nasal product.
- Evaluation of the pre-dispersions (nanosuspensions), and the dry nanoparticles by applying the related tests.
- Performing *in vitro* and *in vivo* comparison studies of the nasal formulation.
- Study the stability of the nasal formulation.

## 3. MATERIALS AND METHODS

### 3.1 Materials

#### *3.1.1 Active pharmaceutical ingredient*

LOR was purchased from Teva (Budapest, Hungary).

#### *3.1.2 Excipients*

Different types of stabilizers were used to prepare the nanosuspensions, and a mucoadhesive agent was used to formulate the nanosuspension into nasal formulations. PVP-K25 was purchased from ISP Customer Service GmBH (Cologne, Germany), Soluplus® and F68 were purchased from BASF (Ludwigshafen, Germany), Tween 80 was purchased from Fluka Chemika (Buchs, Switzerland), HPMC was supplied by Colorcon (Budapest, Hungary), HA was purchased from Gedeon Richter Plc. (Budapest, Hungary), and TRE was purchased from Sigma-Aldrich (New York, USA).

### 3.2 Methods

#### *3.2.1 Determination of QbD elements*

To illustrate the relevant knowledge and information, an Ishikawa diagram was set up. The technical tool used for the RA was LeanQbD® software (QbDWorks LLC, Fremont, CA, USA). The interaction between the elements was described as “high” (H), “medium” (M), or “low” (L). Its dynamism is presented in figures generated by the software.

### *3.2.2 Preparation of loratadine nanosuspension (LNS)*

LNSs were prepared using the precipitation-ultrasonication method. LOR was dissolved in ethanol according to its solubility, while the stabilizer(s) was (were) dissolved in water. For the mixtures of stabilizers, one stabilizer was added to the solvent phase, while the other one was added to the antisolvent phase (**Fig.1**). Both solutions were filtered through a 0.45 μm filter (FilterBio PES Syringe Filter, Labex Ltd., Budapest, Hungary). The fresh-made LOR solution was rapidly introduced into the cool antisolvent under sonication using a UP 200 s Ultrasonic processor (HielscheruUltrasonics GmbH, Germany) and different conditions in terms of energy power, sonication time and sonication temperature. The temperature of sonication was controlled (Julabo F32, JULABO GmbH, Germany). The prepared nanosuspensions were stirred at room temperature for 24 h to remove the organic solvent.

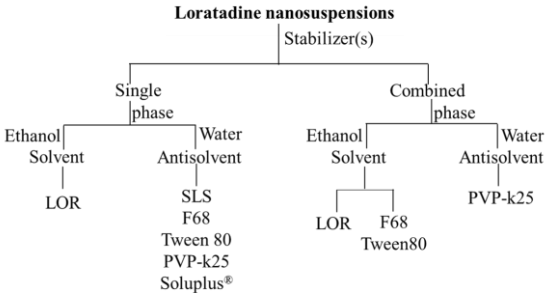


**Figure 1:** Schematic illustration of the contents of the solvent and antisolvent phases of LNSs.

### *3.2.3 Preparation of dry loratadine nanoparticles (DLN)*

The LNSs were dried to obtain solid products to study the physicochemical and investigate the biocompatibility. The LNSs were dried by vacuum oven at 25 °C for 24 h and freeze-drying in a Scanvac, CoolSafe 100-9 Pro type apparatus (LaboGeneApS, Lynge, Denmark). For Freeze-drying, nanosuspensions were lyophilized with 5%, w/v trehalose (TRE) to -40°C.

### *3.2.4 Preparation of nanosuspensions' physical mixtures*

Physical mixtures (PMs) corresponding to the composition of the nanosuspensions were prepared as reference samples by blending LOR with the suitable excipients in a Turbula mixer (Turbula System Schatz; Willy A. Bachofen AG Maschinenfabrik, Basel, Switzerland) using 60 rpm for 10 min.

### *3.2.5 Preparation of loratadine nasal formulations (NFs)*

NFs were prepared from the pre-dispersions by the addition of HA. The final concentrations of the formulations were controlled by dilution with 0.2%, w/v F68. NFs were stored in a refrigerator at 4 °C for 24 h to ensure the complete solvation of the polymer. For comparison, reference samples (REF) were prepared. ***Table I*** shows the final concentrations of LOR and HA in the prepared nasal formulations and corresponding reference samples that contained the same amount of LOR and HA in 0.2%, w/v F68. However, LOR in the reference samples was added without any processing. The REF samples were prepared by mixing raw LOR powder with HA and 0.2% F68 solution, using ULTRA-TURRAX® homogenizer at 5000 rpm for 10 min (GmbH, Germany).

***Table I:*** *Concentrations of LOR and HA (mg mL-1) in the HA-based nasal formulation and reference samples.*

| Sample | LOR (mg $mL^{-1}$) | HA (mg $mL^{-1}$) |
|---|---|---|
| **NF1** | 1 | 1 |
| **NF2** | 1 | 5 |
| **NF3** | 2.5 | 1 |
| **NF4** | 2.5 | 5 |
| **REF1** | 1 | 1 |
| **REF2** | 1 | 5 |
| **REF3** | 2.5 | 1 |
| **REF4** | 2.5 | 5 |

### *3.2.6 Morphology and micrometric characterization of nanosuspensions and dry nanoparticles*

For morphology, SEM (Hitachi S4700, Hitachi Scientific Ltd., Tokyo, Japan) was used applying 10.0 kV high voltage at 10 mA for 10 min. Samples were sputter-coated with gold-palladium (Bio-Rad SC 502, VG Microtech, UK).

The mean particle size (MPS), zeta potential (ZP), and polydispersity index (PDI) of LNSs were measured by laser diffraction using a Malvern Nano ZS zetasizer (Malvern Instrument, UK), using water as the dispersant. 12 parallel measurements were carried out.

### *3.2.7 Structural analysis of the dry nanoparticles*

XRPD was applied using a BRUKER D8 Advance X-ray powder diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) with Cu K $\lambda_1$ radiation ($\lambda$ = 1.5406 Å) and a VÅNTEC-1 detector. Powder samples were scanned at 40 kV and 40 mA, with an angular range of 3° to 40° 2θ, at a step time of 0.1 s and a step size of 0.01°. Eva software was used to calculate the crystallinity index.

FT-IR spectra the samples were obtained by Fourier-transform infrared spectroscopy (Thermo Nicolet AVATAR 330, USA) equipped with the GRAMS/AI ver. 7program. Samples were grounded and compressed into pastilles with 150 mg dry KBr. The pastilles were scanned 128 times at a resolution of 4 $cm^{-1}$in the wavenumber region 4000–400 $cm^{-1}$.

### *3.2.8 Thermal analysis of the dry nanoparticles*

DSC (Mettler Toledo DSC $821^e$, Mettler Inc., Schwerzenbach, Switzerland) was used to obtain the thermograms of the samples. About 3–5 mg of powder was accurately weighed into DSC sample pans, which were hermetically sealed and lid pierced. An empty pan was used as a reference in an inert atmosphere under constant argon purge. The samples were analyzed in the temperature range of 25–300 °C at a heating rate of 5°C $min^{-1}$.

### *3.2.9 Solubility analysis*

Saturation solubility of the samples was investigated by adding excess amounts of the sample into 5 ml of water, ANF, PBS (pH 7.4), or PBS (pH 5.6) at 25ºC. Next, the suspensions were filtered, and the drug concentrations in the filtrate were measured by UV spectroscopy at $\lambda_{max}$ 248 nm.

### *3.2.10* Drug content and dissolution behaviors

LOR contents of the samples were determined by dissolving 10 mg of the dry nanoparticles in 50 ml of 0.1N HCl.

After stirring the solution with a magnetic stirrer (400 rpm) at room temperature for 24 h, it was filtered and analyzed. The concentration was measured spectrophotometrically at 248 nm.

The modified paddle method (USP dissolution apparatus, type II Pharma Test, Hainburg, Germany) was used to characterize the dissolution rates. Concentrations of LOR were measured spectrophotometrically (Unicam UV/VIS Spectrophotometer, Cambridge, UK) at $\lambda_{max}$ 248 nm.

Dialysis bag was used to study the release of LOR from the NFs in ANF media at pH 5.6. 300 mg of the NF and corresponding reference were loaded into a dialysis bag and dialyzed against 100 mL of the dissolution medium at 37 ± 0.5 °C and under 100 rpm paddle speed.

The permeability studies were executed using a vertical Franz diffusion cell system (Logan Instrument Carporation, NJ, USA). 300 mg of NF was placed on the polyvinylidene fluoride synthetic membrane. The actual diffusion surface was 1.72 $cm^2$. Phosphate buffer (PBS, pH 7.4, 37 °C) was used as an acceptor phase (7 ml). The rotation of the stirring bar was set to 300 rpm. The flux (J) of the drug was calculated from the quantity of LOR that permeated through the membrane, divided by the surface of the membrane insert and the duration [mg $cm^{-2}$ $h^{-1}$] using the following equation.

$$J = \frac{m}{At} \qquad (1)$$

The permeability coefficient (Kp, cm $h^{-1}$) was determined from J and the initial concentration of the drug in the donor phase (Cd [mg $cm^{-3}$]):

$$Kp[cm/h] = \frac{J}{Cd} \qquad (2)$$

### *3.2.11 pH of the nasal formulations*

1 mL of the prepared NF was transferred into a 10 mL volumetric flask. The solution was diluted with distilled water. The pH of the resulting solution was determined using a digital pH meter (Inolab, pH 7116, Xylem Analytics Germany GmbH, Germany).

### *3.2.12 Rheological measurements of the NFs*

Rheological measurements were performed at 37 ºC with a Rheostress 1 Haake instrument (Karlsruhe, Germany). The flow curves of the samples were plotted under the shear rate range of 0.01 to 100 $s^{-1}$. This viscosity change, called the bioadhesive viscosity component (ηb), was calculated as follows:

$$\eta_b = \eta_t - \eta_m - \eta_p \qquad (3)$$

Where $\eta_t$ is the viscosity of the combination of NF with mucin, $\eta_m$, and $\eta_p$ are the viscosities of the mucin and NF, respectively. NFs were stirred with mucin (M) for 3h before the measurement. The final concentration of M in the samples was 5% w/w. The viscosity of the NFs and the combination with mucin were measured.

### *3.2.13 In vivo studies of the nasal formulations*

A single-dose *in vivo* studies were designed in male Sprague-Dawley rats weighing 220-250 g. The rats were divided into 4 groups of 4 rats each. Each rat received a dose of 0.5 mg $kg^{-1}$ of LOR. For the first group, 50–62 µL of the selected NF was administered intranasally to each rat via a 100 µL pipette into the nostrils. For the second group, the

rats were nasally given the corresponding REF sample. For the oral dose, the third and fourth groups received the selected NF sample and the corresponding REF sample, respectively. However, the samples were mixed with distilled water to give the exact used dose in a proper volume for oral delivery. 1 mL contained 0.5 mg $kg^{-1}$ of LOR of the samples was administered by gastric lavage.

Blood samples were collected from the tail vein. At predetermined time points, 0.5 mL of blood was withdrawn into Eppendorf tubes containing sodium ethylenediaminetetraacetate. The samples were centrifuged at 1,500 g for 10 min at 5 °C. Separated plasma samples were stored at -80. LOR was isolated from plasma samples by a liquid-liquid extraction procedure by using 10 µL ACN: $H_2O$, (1:1, v/v), 10 µL of 3M NaOH, and 20 µL of d5-Loratadine (d5-LOR) −stable isotope-labeled internal standard (15.0 ng $mL^{-1}$, in ACN:$H_2O$, 1:1, v/v)− as extracting solvents. The quantitative analysis of LOR was performed by using a Waters Acquity I-Class UPLC™ system (Waters, Manchester, UK).

### *3.2.14 Statistical analysis*

The statistical analysis was performed with Prism 5.0 software (GraphPad, San Diego, CA). The results are shown as the mean ± SD. The statistical methods included Student's *t*-test (two-group comparison). A probability (P) of less than 0.05 was considered statistically significant (*P < 0.05).

### *3.2.15 Stability*

Stability studies were carried out by visual inspection. Stability was observed at temperatures of 4 ºC and 25 ºC for 6 weeks. Moreover, the formulations were evaluated for particle size, polydispersity index, zeta potential, and drug content.

## 4. RESULTS

### 4.1 QbD methodology of LOR nanosuspension-based NFs

For pre-formulation, an Ishikawa diagram set up, including all the parameters influencing the desired nanosuspension-based nasal formulation containing LOR as the active agent (**Fig.2**).

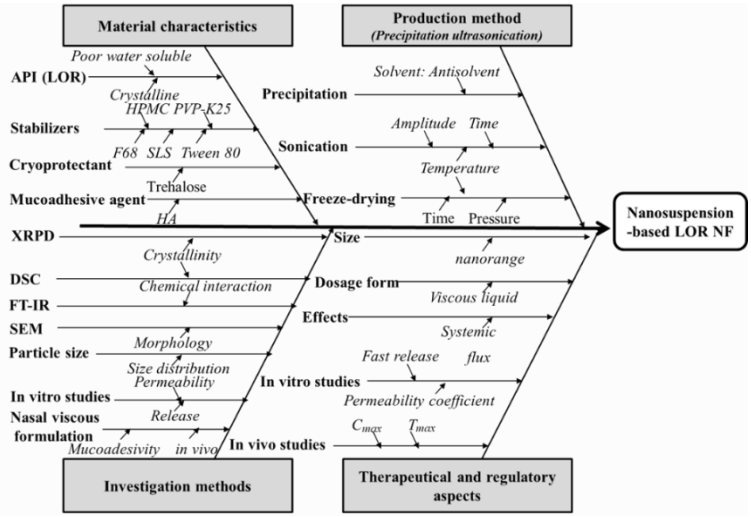


**Figure 2:** *Ishikawa diagram illustrating the parameters influencing the quality of the NF containing nanosized LOR.*

The next step was to select the elements of QTPPs, CQAs, CMPs, and CPPs for the aimed nasal product. For adaptation to the QbD-based development principles, QTPP and the required CQAs were defined.

RA reveals the interdependence rating between the QTPP and CQAs, and between CQAs and the CPPs-CMPs. Accordingly, particle size, polydispersity index, zeta potential, structure, stability, solubility, and dissolution rate were highly influenced by drug concentration, stabilizer type and concentration, and sonication time, power, and temperature.

### 4.2 Selection of process parameters to prepare LNSs and DLNs

For process optimization, the drug amount and the stabilizer's type and concentration were fixed at 100 mg and 0.2% w/v of F68, respectively. Additionally, various solvent: antisolvent ratios, sonication temperatures, sonication times, and sonication powers were applied at fixed freeze-drying conditions (**Fig.3**).

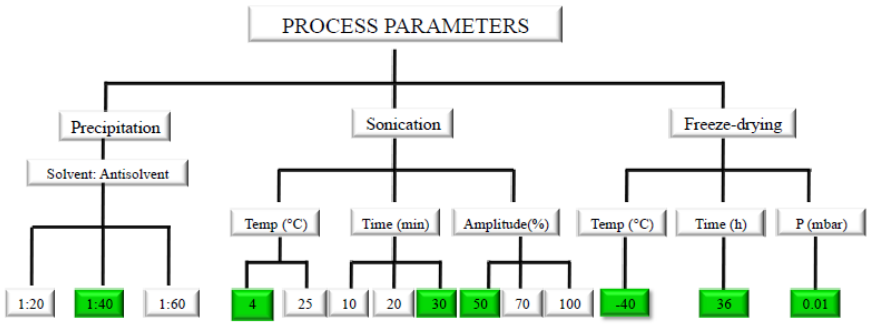


**Figure 3**: *Critical process parameters for the preparation of LNS and DLNs.*

In summary, LNSs suitable for further processing were prepared using the following process parameters: 30 min sonication time, 50% amplitude of sonication power, 4 ºC for the sonication temperature, and a solvent:antisolvent ratio of 1:40.

### 4.3 Effects of material parameters on particle size and stability of LNSs

Different drug concentrations and various stabilizer types and concentrations were used to prepare LNSs (Table II). Using of HPMC, SLS, or PVP-K25 alone were not able to produce LNSs. On the other hand, Tween 80 and F68 were suitable to produce LNSs when they were used on their own. Different concentrations of F68 as a single stabilizer yielded different MPSs with increasing diameters as concentration increased. Soluplus® produced LNSs of particle size smaller than the commonly used stabilizers. Unlike F68, the particle size decreased with increased Soluplus® concentration. Higher concentrations of Soluplus® could stabilize the LNS more effectively due to weak Ostwald ripening as the drug will diffuse slowly from the formed micelles.

Based on particle size, nanosuspensions with 0.6% Soluplus®, and 0.2% w/v F68 either as a single stabilizer or as a mixture with PVP-K25 at 1:1 or 1:2 ratios (LNS5, LNS12, LNS13, and LNS18) were selected to be dried and further analyzed to evaluate the morphology, thermal, structure, solubility, and dissolution characteristics.

Aggregation of the selected LNSs did not occur for 1 week upon storage at 4°C, and nanoscale size was preserved. However, MPS increased for all the selected samples compared to the MPS measured on the day of preparation, a

particle size increment of 28.5–30 nm must be considered overtime.

Other prepared DLNs were easily redistributed to their original volume at nanosized range with accepted PDI and higher ZP than corresponding nanosuspensions, probably due to enhanced specific interaction between LOR and the polymeric stabilizers during drying and hence stability.

***Table II***: *Mean particle size (MPS), polydispersity index (PDI) and zeta potential (ZP) for LOR and LNSs (Mean ± SD).*

| Sample | LOR (mg) | Stabilizer type | Stabilizer concentration (% w/v) | MPS (nm) | PDI | ZP (mV) |
|---|---|---|---|---|---|---|
| **LOR** | 100 | - | - | 4607.5±41.7 | 0.71±0.18 | -7.7±5.28 |
| **LNS1** | 100 | PVP-K25 | 0.2 | 4900±71.98 | 0.98±0.028 | -13.4±4.02 |
| **LNS2** | 100 | HPMC | 0.2 | 4212±14.14 | 0.767±0.18 | -11.9±4.51 |
| **LNS3** | 100 | SLS | 0.2 | 1496.3±17.45 | 0.414±0.11 | -54±7.75 |
| **LNS4** | 100 | Tween 80 | 0.2 | 414.9±9.02 | 0.217±0.03 | -23±6.51 |
| **LNS5** | 100 | F68 | 0.2 | 246.5±1.83 | 0.133±0.03 | -6.5±3.98 |
| **LNS6** | 100 | F68 | 0.4 | 288.3±37.33 | 0.104±0.01 | -6.4±4.45 |
| **LN7** | 100 | F68 | 0.6 | 325.4±28.20 | 0.198±0.01 | -12.1±5.91 |
| **LNS8** | 100 | PVP-K25+SLS | 0.2+0.2 | 589.3±12.66 | 0.226±0.03 | -58.7±8.54 |
| **LNS9** | 100 | F68+SLS | 0.2+0.2 | 557.4±31.47 | 0.196±0.03 | -67.2±8.14 |
| **LNS10** | 50 | F68+PVP-K25 | 0.2+0.2 | 306.7±14.97 | 0.158±0.11 | -27.8±5.08 |
| **LNS11** | 75 | F68+PVP-K25 | 0.2+0.2 | 276.5±2.69 | 0.108±0.02 | -4.8±4.11 |
| **LNS12** | 100 | F68+PVP-K25 | 0.2+0.2 | 253.4±1.27 | 0.123±0.01 | -11.1±4.89 |
| **LNS13** | 100 | F68+PVP-K25 | 0.2+0.4 | 265.6±20.79 | 0.122±0.03 | -18.1±3.85 |
| **LNS14** | 100 | F68+PVP-K25 | 0.2+0.6 | 307.25±7.28 | 0.166±0.01 | -23.6±5.07 |
| **LNS15** | 100 | Tween80+PVP-K25 | 0.2+0.2 | 423.4±15.06 | 0.202±0.02 | -22.9±4.39 |
| **LNS16** | 100 | Soluplus® | 0.2 | 220.35±5.3 | 0.25±0.0 | -21.5±5.59 |
| **LNS17** | 100 | Soluplus® | 0.4 | 178.7±6.5 | 0.12±0.02 | -19.7±4.85 |
| **LNS18** | 100 | Soluplus® | 0.6 | 168.3±6.5 | 0.16±0.03 | -16.5±6.59 |

## 4.4 Morphology of the DLNs

Raw LOR showed irregular crystal shapes with a particle size larger than 5 µm with aggregation. DLN5, DLN12, and DLN13 were characterized by short rod shape particles at the nanoscale, while Soluplus® containing sample (DLN18) had spherical particles at the nanosized scale embedded within the carriers (**Fig.4**).

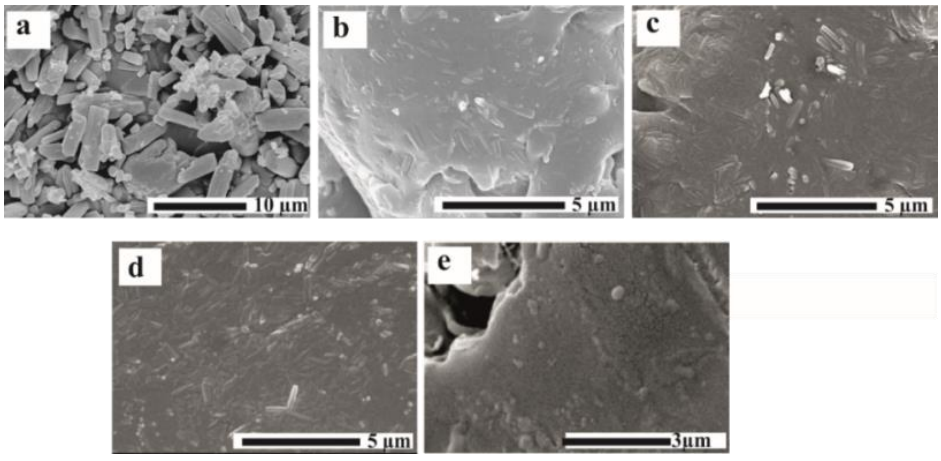


**Figure 4***: SEM images of (a) raw LOR, (b) DLN5, (c) DLN12, (d) DLN13, and (e) DLN18.*

### 4.5 Thermal and structural analysis of the DLNs

The thermograms of DLNs (**Fig.5a**) confirmed the absence of the melting point of LOR (at 135.5 ºC). Moreover, it exhibited thermal events at 90, 211, and 270 °C due to the glass transition temperature, melting of trehalose, and decomposition of trehalose, respectively.

For the XRPD analysis (**Fig.5b**), the PMs showed the characteristic crystalline diffraction peaks of LOR. However, the DLNs showed the halo and the diffused pattern typical of amorphous material. The degree of crystalline index confirmed the amorphous form of the LOR in these samples (37, 37, 18.1, and 27%for DLN5, DLN12, DLN13, and DLN18, respectively).

FT-IR spectra showed that the PMs spectra had the characteristic peaks of pure LOR, indicating negligible interactions between the LOR and the excipients. On the other hand, DLN5, DLN12, DLN13, and DLN18 showed significant differences at 3532, 2900-2982, 1700, and 997–1171 $cm^{-1}$. These shifts could be ascribed to the interaction of LOR with the excipients during freeze-drying (**Fig.6**).

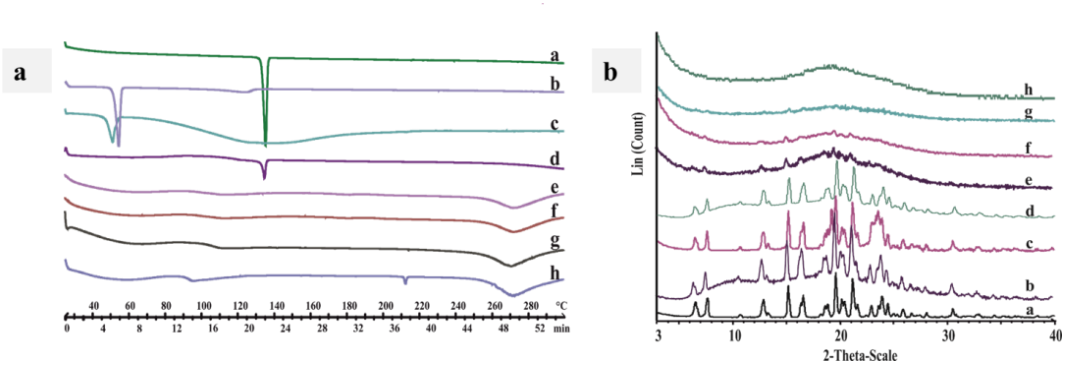


**Figure 5: (a)** *DSC thermograms and* **(b)** *XRPD diffractograms of (a) raw LOR, (b) PM1 (1.25:1 weight ratio of LOR: F68), (c) PM2 (1.25:1:1 weight ratio of LOR:F68:PVP-K25), (d) PM3 (1:2.4 weight ratio of LOR:Soluplus®), (e) DLN5, (f) DLN12, (g) DLN13, and (h) DLN18.*

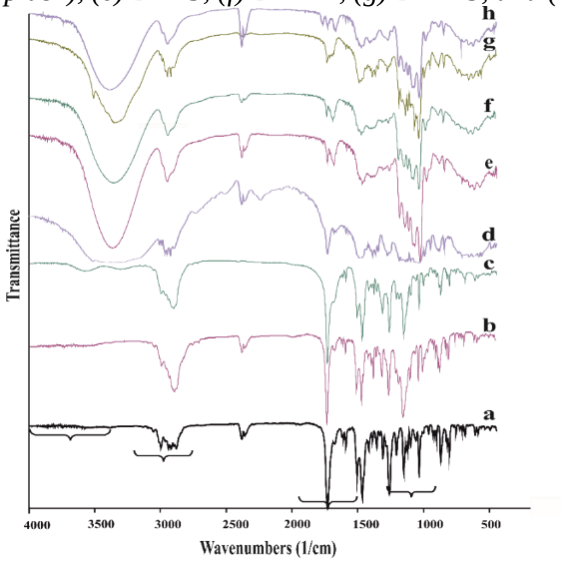


**Figure 6:** *FT-IR spectra of* ***(a)*** *raw LOR,* ***(b)*** *PM1 (1.25:1 weight ratio of LOR: F68), (c) PM2 (1.25:1:1 weight ratio of LOR:F68:PVP-K25), (d) PM3 (1:2.4 weight ratio of LOR:Soluplus®), (e) DLN5, (f) DLN12, (g) DLN13, and (h) DLN18.*

### 4.6 Solubility and *in vitro* release from dried nanoparticles

Compared to pure LOR, DLNs showed enhanced saturation solubility in water and PBS of pH 7.4. The water solubility of nanoparticles was increased by approximately 5.5, 8.6, and 15.4-fold for DLN5, DLN12, and DLN13, respectively. On the other hand, solubility in PBS of pH 7.4 was enhanced by 9.3, 8.0, and 8.6-fold for DLN5, DLN12, and DLN13. Moreover, DLN18 showed a 59.39 ±5.18 μg $mL^{-1}$ solubility of LOR in PBS (pH 7.4), this means 121-fold enhanced solubility compared to LOR.

DLNs showed higher drug release than pure LOR and PMs. 30–42% of the drug was detected to be released in the first 10 min from the DLN5, DLNS12, and DLN13. On the other hand, about 57% of LOR was released from the Soluplus®-based dry nanoparticles (DLN18) in the first 15 min and 80% within 2 h (**Fig.7**).

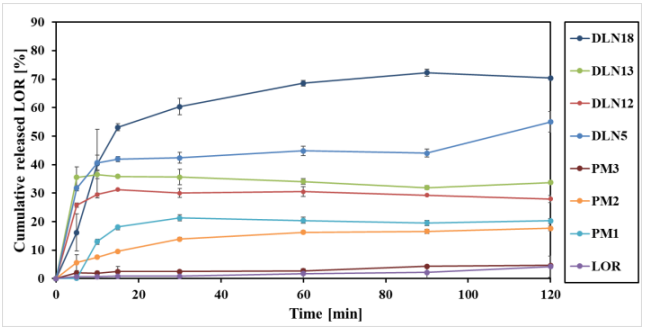


***Figure 7:*** *Dissolution behaviors of LOR, PM1, PM2, PM3, DLN5, DLN12, DLN13, and DLN18 at PBS, pH 7.4 (Mean ± SD, n=3).*

### 4.7 Preparation of the LOR pre-dispersion for the nasal formulations

Based on the previous experiments for the preparation and characterization of LNS, LNS5 was selected as a base to prepare the pre-dispersion for the nasal formulations. However, the drug content was increased. Accordingly, material and process parameters were set as 200 mg $mL^{-1}$ of LOR concentration of in the solvent phase, 0.2% w/v F68 as an antisolvent phase, 1:40 (mL:mL) of solvent:antisolvent. Moreover, the sonication process was set for sonication time of 30 min, sonication temperature of 4 ºC, and sonication power of 50% amplitude.

### 4.8 Characterization of LOR pre-dispersion

The morphological and structural analyses have demonstrated that LOR was produced in the nano-range (312 nm) as a homogenous nanosuspension while it preserved the crystalline state of the drug (**Fig.8**).

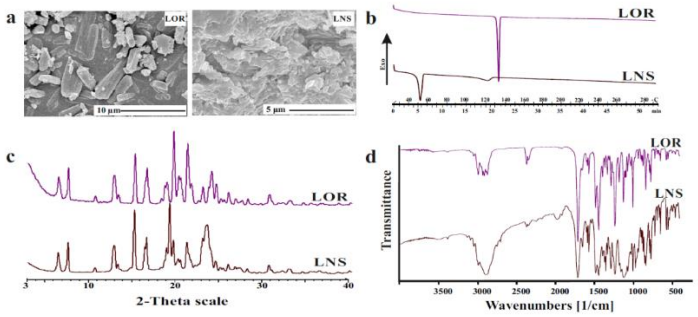


**Figure 8:** *Vacuum dried LNS characterization of* ***(a)*** *SEM images,* ***(b)*** *DSC thermograms,* ***(c)*** *XRPD diffractograms, and* ***(d)*** *FTIR spectra.*

### 4.9 Characterization of the nasal formulations

The prepared NFs appeared as viscous formulations. The samples showed drug content higher than 90%. The pH of the samples was in the range of 6.3–6.4, hence within the acceptable range for nasal administration (pH of the nasal mucosa is 4.5- 6.5). The MPS and ZP were increased by the addition of HA. The MPS of LOR in NF1, NF2, NF3, and NF4 (***Table I***) was 327.2 ± 8.23, 437.27 ± 28.6, 341.6 ± 11.84, and 450. 633 ± 24.3 nm, respectively. Their respective PDI values were 0.249, 0.314, 0.254, and 0.264, respectively. The ZP values were -55.1 ± 5.67, -50.3 ± 3 ± 6.68, -45.9 ± 6.36, and -52.2 ± 6.91 mV for NF1, NF2, NF3, and NF4, respectively.

### 4.10 Rheological properties of NFs

The NFs showed a shear thinning-flow (pseudoplastic) (**Fig.9a**). The rheological behaviors of the NFs were similar to the corresponding blank solutions that contained 1mg $mL^{-1}$ and 5 mg $mL^{-1}$ of HA in 0.2% w/v F68 noted as blank1 and blank5, respectively.

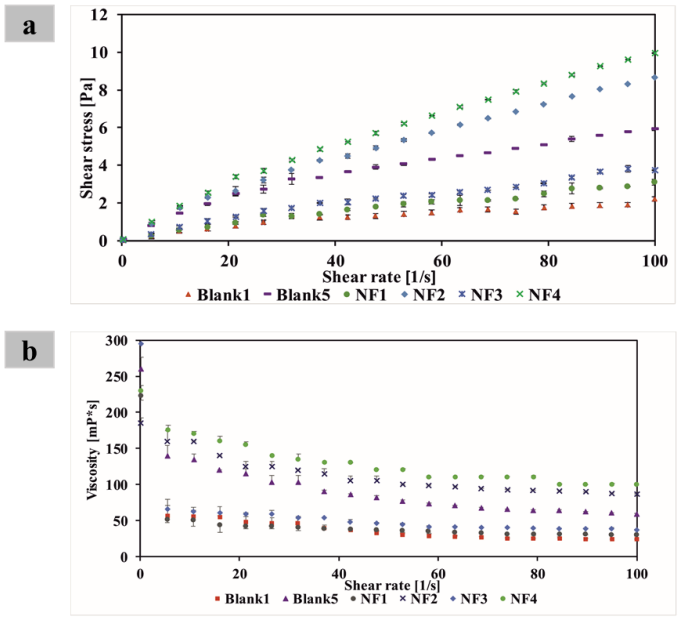


**Figure 9*:(a)*** *The flow curves, and* ***(b)*** *the apparent viscosity of the NFs, blank1, and blank5 samples at 37 ºC (Mean ± SD, n=3).*

The apparent viscosity of the NFs was decreased by increasing the shear rate, which is typical for sodium hyaluronate solutions (**Fig.9b**) (Krause et al., 2001). However, the reduced particle size of LOR showed higher viscosity than the blank samples. Therefore, the nanosized LOR improved the viscosity of blank solutions. Apart from this, the viscosity of the formulations was related to the used HA polymer concentration. 1 mg $mL^{-1}$ containing NFs (NF1 and NF3) showed lower values than 5mg $mL^{-1}$ containing NFs (NF2 and NF4).

### 4.11 Mucoadhesion of the NFs

The bioadhesive viscosity component, synergism parameter, was calculated from the average viscosity values (**Fig.10**). The blanks showed mucoadhesive properties, depending on the concentration of the

sodium hyaluronate. The values of the bioadhesive viscosity were 0.6 and 46.5 mPa*s for blank1 and blank5, respectively. The negative values $\eta_b$ of REF1 and REF3 could be related to the insufficient amount of HA to interact with the mucin.

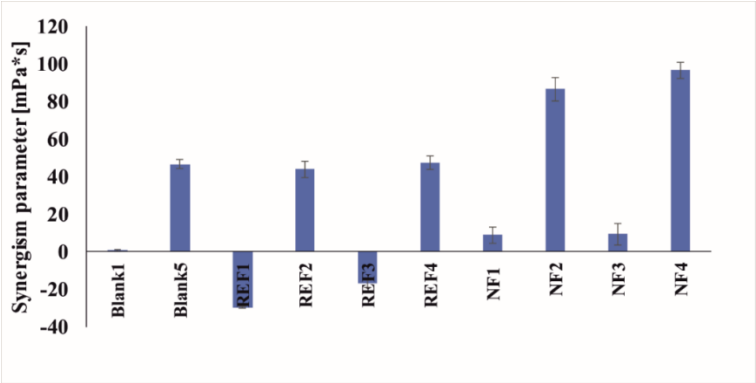


**Figure 10**: *Calculated synergism parameters of blanks, REF, and NF samples at a shear rate of 100 $s^{-1}$ and 37 ºC (Mean ± SD, n=3).*

NF4 that showed the highest mucoadhesive parameter. Therefore, it was selected for further studies.

### 4.12 *In vitro* and *in vivo* studies of NF4

NF4 showed an enhanced drug release compared to the reference sample (**Fig.11a**). Approximately 77% of the drug was released from NF4 within the first 15 min compared to 10% from the reference sample. These discrepancies in dissolution rates could be related to the nanosizing effects, as small particles produced a higher surface area than the microparticles. The nanoparticles showed a significantly increased J compared to REF4 (24.73 ± 3.2 and 1.49 ± 1.03 µg $cm^{-2}$ $h^{-1}$, respectively). The permeability coefficient (Kp) of NF4 also showed a higher value than REF4. Kp values were 0.082 and 0.017 cm $h^{-1}$, respectively (**Fig.11b**).

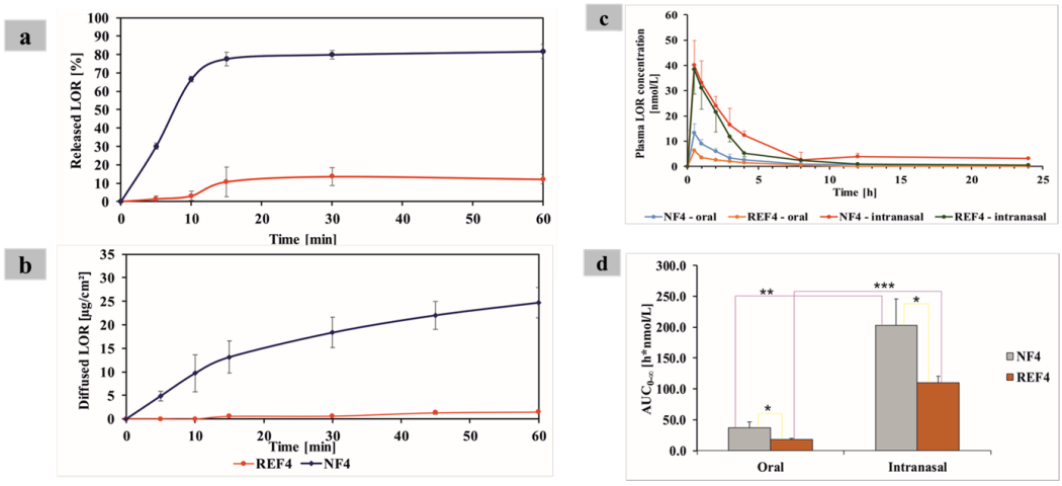


**Figure11**:***(a)*** *Dissolution profile,* ***(b)*** *In vitro permeability of NF4 and REF4 in ANF media at 37 ºC (Mean ± SD, n=3),* ***(c)*** *Plasma concentration of LOR (nmol $L^{-1}$), and* ***(d)*** *AUC 0– ∞ (h nmol $L^{-1}$) after nasal and oral administration of NF4 and REF4 (Mean ± SD, n=3).*
*(*, P=0.02; **, P=0.003, ***, P=0.0003)*

The plasma levels after intranasal administration were higher than the oral delivery of the samples (**Fig.11c**). The $C_{max}$ values were 6.39, 13.29, 38.36, and 39.99 nM for REF4-oral, NF4-oral, REF4-nasal, and NF4-nasal, respectively (***Table III***).

**Table III:** Pharmacokinetics parameters of LOR concentration in plasma after administration of NF4 and REF4 using oral and intranasal administration (Mean ± SD, n = 4).

| | **Oral** | | **Intranasal** | |
|---|---|---|---|---|
| | **REF4** | **NF4** | **REF4** | **NF4** |
| **$AUC_{0-\infty}$ [h nmol $L^{-1}$]** | 17.81±1.96 | 36.59±9.79 | 110.35±10.41 | 202.71±43.31 |
| **$C_{max}$ [nM]** | 6.39±2.21 | 13.29±5.72 | 38.36±9.78 | 39.99±14.18 |
| **$k_e$ [$h^{-1}$]** | 0.24±0.03 | 0.24±0.03 | 0.24±0.09 | 0.12±0.013 |

The relative bioavailability of the intranasal delivered NF4 was 1.84-fold compared to the REF4 and 5.54-fold compared to the oral delivered sample i.e. NF4-oral. These findings provide evidence that nasal administration enhanced the bioavailability of LOR. Moreover, the nanoparticles are practical to improve the delivery of LOR through the nasal route (**Fig.11d**).

### 4.13 Stability of NF4

There was no significant change in terms of physical appearance and viscosity. Furthermore, no particle precipitation occurred over 4 weeks for the samples kept at 4 ºC. The drug content of NF4 samples after the storage period was 89.48 ± 3.6%. The mean particle size of LOR nanosuspension in NF4 was 425.5. Moreover, the NF4 showed a PDI of 0.37 and ZP of – 42.6. The stability of the formulation could be related to the high zeta potential and the viscosity of the formulation that kept the LOR nanoparticles separated and homogeneously distributed through the matrix.

## 5. CONCLUSIONS

- QbD was implemented to define the QTPP of the final nasal formulation as well as the CQAs, CPMs, and CPPs for the preparation of the LOR nanosuspensions. The RA was used to evaluate the influential effects of the CMPs and CPPs according to the required CQAs.
- For the preparation of nanosuspensions using the precipitation- assisted ultrasonication method, particle size, polydispersity index, zeta potential, dissolution rates were set as the CQAs of high interest. On the other hand, the CMPs of drug amount, stabilizer(s) type and concentration, and solvent: antisolvent ratio, as well as CPPs of sonication time, temperature, and power, were optimized.
- Nanosuspension has been used as a pre-dispersion for the preparation of nasal formulation as a simple and straightforward strategy. NF4 formulation that contained 2.5 mg $mL^{-1}$ of loratadine and 5 mg $mL^{-1}$ sodium hyaluronate showed enhanced rheological behaviors, where nanosizing had a main effect in the mucoadhesive properties. NF4 showed enhanced dissolution in an artificial nasal fluid. Besides, higher diffusion and permeability coefficient compared to the unprocessed loratadine.
- The *in vivo* studies showed the superiority of nasal delivery over the oral administration. Moreover, the nanoparticles showed higher AUC0–∞ compared to the unprocessed LOR.
- Nanosuspension-based nasal formulation (NF4) showed good stability over 6 weeks of storage period at 4 ºC.

# 6. NOVELTY AND PRACTICAL ASPECTS

- The novelty and power of the presented work based on its ability to control and compromise different aspects from analyzing the literature to select the route of administration to produce LOR in a new dosage form that has not been studied and developed before.
- Applying QbD rationalized the selection of the methodologies and the route of the administration, significantly improved the targetability of getting optimized formulations in the voice of predefined quality and safety.
- Optimization of critical parameters to produce LOR's nanosuspension is considered a significant step toward extending the application of precipitation-assisted ultrasonication methods to formulate different APIs as nanosuspension-based dosage forms. By these findings, this method can compete with the top-down one in the development of potential products for the market.
- A combination of the nanosuspension and the simple addition of a mucoadhesive agent could suggest a promising platform for the nasal delivery of various poorly water-soluble drugs.
- A novel formulation of LOR has been developed based on the nanosuspension of the drug. The prepared nasal formulation showed an improved bioavailability of LOR. Therefore, this formulation could offer new possibilities for the delivery of LOR as a new dosage form.
- Developing nanosuspension-based nasal formulation with an improved bioavailability compared to oral delivery could boost the chances for the nasal formulations to enter the market.
- The applicability of nanosuspensions as a nasal delivery system to the systemic circulation is a new approach in pharmaceutical technology.

## PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

1. **A. Alshweiat**, G. Katona, I. Csóka, R. Ambrus, *Design and characterization of loratadine nanosuspension prepared by ultrasonic-assisted precipitation*, Eur. J. Pharm. Sci. (2018) 1–34. doi:10.1016/j.ejps.2018.06.010.

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2. **A. Alshweiat**, R. Ambrus, I. *Csóka QbD based control strategy of loratadine nanosuspensions and dry nanoparticles stabilized by soluplus®*, Farmacia. 67 (2019) 729–735. doi:10.31925/farmacia.2019.4.23

   **IF: 1.527 (2018), Q2**

3. **A. Alshweiat**, R. Ambrus, I. Csóka. *Intranasal Nanoparticulate Systems as Alternative Route of Drug Delivery*, Curr. Med. Chem. 26 (2019) 1–34. doi:10.2174/0929867326666190827151741

   **IF: 3.894 (2018), Q1**

4. **A. Alshweiat**, I. Csóka, F. Tömösi, T. Janáky, A. Kovács, R. Gáspár, A. Sztojkov-Ivanov, E. Ducza, Á. Márki, P. Szabó-Révész, R. Ambrus., *Nasal delivery of nanosuspension-based mucoadhesive formulation with improved bioavailability of loratadine: preparation, characterization, and in vivo evaluation.*, Eur. J. Pharm. Sci. (2018) 119166. doi: 10.1016/j.ijpharm.2020.119166.

   **IF: 4.213 (2018), Q1**

## OTHER PUBLICATIONS

1. R. Ambrus, **A. Alshweiat**, I. Csóka, G. Ovari, A. Esmail, N. Radacsi, *3D-printed electrospinning setup for the preparation of loratadine nanofibers with enhanced physicochemical properties*, Int. J. Pharm. 567– (2019) 118455. doi:10.1016/j.ijpharm.2019.118455.

   **IF: 4.213 (2018), Q1**

## PRESENTATIONS RELATED TO THE THESIS

***Poster presentations:***

1. **Alshweiat, A**., Ambrus, R., Katona, G., Csóka, I; *Preparation and evaluation of loratadine nanosuspension by using precipitation ultrasonication technology*. 7th BBBB International Conference of Pharmaceutical Sciences. October 2017. Balatonfüred, Hungary.
2. **Alshweiat, A**., Katona, G., Csóka, I., Ambrus, A; *Incorporation of loratadine into intranasal delivery system*. BioNanoMed. April 2018**.** Graz, Austria.
3. **Alshweiat, A.**, Ambrus, A., Katona, G., Csóka I; *Preparation and investigation of loratadine nanosuspension stabilized by soluplus® to improve physico-chemical properties*. EUFEPS. May 2017. Athens, Greece.

***Verbal presentations:***

4. **Alshweiat, A**., Ambrus, R., Csóka, I; *Implementation of QbD approach for development of nanosized intranasal products*. 1st Young Technologists' Forum. April 2018. Budapest, Hungary.
5. **Alshweiat. A**., Csóka, I., Ambrus, A; *Development of Sodium Hyaluronate-based Formulations Loaded with Nanosuspension for Nasal Delivery of Loratadine: Simplicity of Preparation and Application*. I. Symposium of

Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Affairs. January 2019. Szeged, Hungary

6. **Alshweiat, A**., Csóka, I., Ambrus, R; *Nanosystems for improved physicochemical properties of poorly water-soluble loratadine*. II. Symposium of Young Researchers on Pharmaceutical Technology, Biotechnology and Regulatory Affairs. January 2020. Szeged, Hungary

**PRESENTATIONS NOT RELATED TO THE THESIS**

1. Ambrus, R., **Alshweiat, A**., Csóka,I., Óvári, G., Radacsi, N; *Characterization of electrospun loratadine-PVP composite nanofibers prepared by a 3D-printed electrospinning apparatus*. CESPT. August 2018. Szeged, Hungary

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